



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : A61K 31/00	A2	(11) International Publication Number: WO 00/45800 (43) International Publication Date: 10 August 2000 (10.08.00)
(21) International Application Number: PCT/EP00/00938 (22) International Filing Date: 2 February 2000 (02.02.00) (30) Priority Data: 60/118,295 2 February 1999 (02.02.99) US 60/118,235 2 February 1999 (02.02.99) US 60/118,282 2 February 1999 (02.02.99) US (71) Applicant (for all designated States except US): K.U. LEUVEN RESEARCH & DEVELOPMENT [BE/BE]; Groot Begijnhof, Benedenstraat 59, B-3000 Leuven (BE). (72) Inventors; and (75) Inventors/Applicants (for US only): WAER, Mark, Jozef, Albert [BE/BE]; Waversebaan 263, B-3001 Heverlee (BE). HERDEWIJN, Piet, André, Maurits, Maria [BE/BE]; Olivierstraat 21, B-3111 Rotselaar/Wezemaal (BE). PFLEIDERER, Wolfgang, Eugen [DE/DE]; Lindauerstrasse 47, D-78464 Konstanz (DE). (74) Agent: PRINS, Hendrik, Willem; Arnold & Siedsma, Sweelinckplein 1, NL-2517 GK The Hague (NL).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: IMMUNOSUPPRESSIVE EFFECTS OF PTERIDINE DERIVATIVES		
<div style="display: flex; justify-content: space-between; align-items: center; margin-top: 10px;"> <div></div> <div>(I)</div> </div>		
(57) Abstract The invention relates to a pharmaceutical composition for the treatment of autoimmune disorders and/or for the treatment or prevention of transplant-rejections comprising a pteridine derivative of general formula (I) especially a lumazine optionally combined with a second active agent.		

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IMMUNOSUPPRESSIVE EFFECTS OF PTERIDINE DERIVATIVES

The invention relates to a pharmaceutical composition for the treatment of autoimmune disorders and/or the treatment or prevention of transplant-rejections comprising pteridine derivatives.

5 The invention further relates to combined pharmaceutical preparations comprising one or more pteridine derivatives and one or more known immunosuppressant, and to a group of novel pteridine derivatives as such.

10 Further the invention is also related to a method for the treatment of autoimmune disorders and/or of transplant-rejections.

 Several pteridine derivatives are known in nature and used in the preparation of medicines, for example as
15 described in EP-A-108 890. Other medical uses of derivatives of pteridine are described in WO 95-31987 as NO-synthase inhibitors, for example for the treatment of diseases caused by a high nitrogen monoxide level. Further, WO-95-32203 describes also the use of
20 tetrahydropteridine derivatives as NO-synthase inhibitors.

 Both above-mentioned WO publications disclose also the use of these specific pteridine derivatives in the treatment of pathologically low blood pressure, in
25 particular septic shock and combined with cytokines in tumor therapy and in transplant-rejection diseases.

 Although some of these pteridine derivatives are claimed as potentially active for the treatment of transplant-rejection diseases, direct evidence for their
30 effectiveness is lacking. Thus there still is a need for specific and highly active immunosuppressive compounds, in particular immunosuppressive compounds active in the cosignal pathway.

 A first object of the invention is to provide a
35 pharmaceutical composition having high immunosuppressive activity. Another object of the invention is to provide a

combined immunosuppressive preparation which causes a superadditive effect, comprising a pteridine derivative of the invention and other known immunosuppressants.

Another further object of the invention is to
5 provide immunosuppressive compounds, which are active in a minor dose, in order to decrease the considerable treatment costs.

Known immunosuppressive compounds are for example cyclosporine A, substituted xanthines, tacrolimus
10 (FK 506), rapamycin (RPM), leflunomide, mofetil, adrenocortical steroids, cytotoxic drugs and antibody preparations.

The immunosuppressive effect of cyclosporine A (CyA) is already known since 1972. However, due to its
15 nephrotoxicity and several other side effects CyA has not been able to establish itself as the optimal and final drug of choice.

Methylxanthines, for example pentoxifylline (PTX), are known having immunosuppressive effects in
20 vitro.

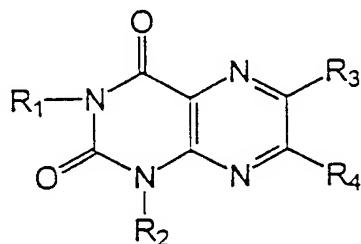
Recently (Lin Y. et al, Transplantation 63 (1997) it has been found that the co-medication of an immunosuppressive compound such as cyclosporine A (CyA) or FK506 or RPM (rapamycin) with a methylxanthine
25 derivative, in particular A802715 (7-propyl-1(5-hydroxy-5-methylhexyl)-3-methylxanthine) leads to a superadditive increase in the immunosuppressive action.

Likewise, other substituted, in particular substituted 8-phenylxanthines have been found to possess
30 immunosuppressive effects in vitro (application EP 98.201323.7).

The present invention relates in particular to the application of a group pteridine derivatives and their pharmaceutical salts, possessing unexpectedly
35 desirable pharmaceutical properties, i.e. are highly active immunosuppressive agents.

The invention demonstrates the

immunosuppressive effects of ³pharmaceutical composition
for the treatment of autoimmune disorders and/or for the
treatment or prevention of transplant-rejections
comprising a pteridine derivative of general formula:



wherein:

R₁ and R₂ are independently hydrogen; aliphatic
saturated or unsaturated; straight or branched carbon
chain with 1 to 7 carbon atoms; substituted or
unsubstituted aryl or alkylaryl substituents, whereby the
carbon atoms may be oxidized represented by alcohol or
carbonyl function or carboxylic acids and their esters;

20

R₃ and R₄ are independently hydrogen, hydroxyl,
halogen, alkyl, haloalkyl, alkoxy, wherein alkyl and the
alkyl group may be branched or straight and contains one
or four carbon atoms, formyl and derivatives such as
hydroxylamino conjugates and acetals, cyano, carboxylic
acids and carboxyl acid derivatives such as esters and
amides, sulfhydryl, amino, alkylamino, cycloalkylamino,
alkenylamino, alkynylamino, benzylamino,
hydroxylalkylamino, morfolinoalkylamino, fenylhydrazino,
morfoline, piperidine, mercaptobenzyl, mercaptoalkyl,
cysteinyl ester, styryl, substituted or unsubstituted
aromatic ring; aromatic or heterocyclic substituent
substituted with an aliphatic spacer between the
pteridine ring and the aromatic substituent of 1 to 4
carbon atoms, whereby said spacer may contain an alcohol
function, carbonyl function, halogen, ether, and may be
saturated or unsaturated; branched or straight, saturated
or unsaturated aliphatic chain of 1 to 7 carbon atoms

which may contain one or more functions chosen from the group comprising carbonyl, alcohol, ether, carboxyester, nitro, thioalkyl, halogen or a pharmaceutically acceptable salt thereof; and

5

X and Y are independently oxygen or sulfur or a pharmaceutical salt thereof, and a pharmaceutically acceptable carrier.

Preferred pteridine derivatives comprising
10 compositions are given in claims 2-9. Particularly preferred are the compositions according to claim 10.

The invention further relates to a combined preparation having synergetic effects containing 1) cyclosporine A, substituted xanthines, tacrolimus
15 (FK506), Rapamycin (RPM), Leflunomide, Mofetil, adrenocortical steroids, cytotoxic drugs and antibody compositions and 2) at least one pteridine derivative of formula (I) defined above, and optionally a pharmaceutical excipient, for simultaneous, separate or
20 sequential use in (auto)immune disorders and/or in the treatment of transplant-rejections.

The invention further relates to a method for treating auto-immuno disorders or transplant-rejections in a subject by administering an effective amount of a
25 pharmaceutical composition of claims 1-11, to the compounds as such as defined above, to the use of these compounds for the treatment of autoimmuno disorders and/or the treatment and/or prevention of transplant rejections, and to a method for selecting potent
30 immunosuppressive agents based on the determination of the three parameters MLC, ACD₃ and ACD₂₈.

Hereunder the effects of the pteridine derivatives on the lymphocyte activation are elucidated and are compared with standard reference compounds (see
35 table I, compound 4, 6, 7, 11, 13, 19, 20, 21, 22, 25, 26, 28, 30, 34, 35).

Table I summarizes the tested compounds. These pteridine derivatives were obtained as follows:

6-Bromomethyl-1,3-dimethylillumazine:

To a solution of 1,3,6-trimethylillumazine [1] (2.06 g, 0.01 moles) in glacial AcOH (60 ml) was added dropwise bromine (3.2 g, 0.02 moles) in AcOH (10 ml) and then
5 heated under reflux for 1 hour. After cooling was evaporated, the residue dissolved in CHCl_3 (100 ml), washed with H_2O (3 x 70 ml), the organic layer dried over Na_2SO_4 and again evaporated. The residue was purified by silica gel column chromatography starting with
10 toluene/EtOAc 9/1 to elute first 6-dibromomethyl-1,3-dimethylillumazine (1.49 g, 41%) and followed by toluene/EtOAc 4/1 to get 6-bromomethyl-1,3-dimethylillumazine. Yield: 1.2 g (42%). M.p. 228°C (decomp.). UV (MeOH): 244 (4.16); [264 (400)]; 337
15 (3.86).

7-Bromomethyl-1,3-dimethylillumazine:

Analogous to the preceding procedure with 1,3,7-trimethylillumazine [1] (2.06 g, 0.01 moles) and bromine
20 (3.2 g, 0.02 moles) by heating for 2 hours. Isolation by silica gel column chromatography with toluene/EtOAc 9/1 to elute first 7-dibromomethyl-1,3-dimethylillumazine (2.07 g, 57%) and second 7-bromomethyl-1,3-dimethylillumazine. Yield: 0.97 g (34%). M.p. 165-166°C. UV (MeOH): 241
25 (4.23); 338 (4.02).

1,3-Dimethylillumazin-6-ylmethyl-triphenylphosphonium bromide:

To a suspension of 6-bromomethyl-1,3-dimethylillumazine
30 (1.0 g, 3.5 mmoles) in toluene (20 ml) triphenylphosphane (1.1 g, 4.2 mmoles) was added and then heated at 80°C in an oilbath with stirring for 8 hours. After cooling the precipitate was collected, washed with EtOAc and dried at 100°C to give 1.8 g (94%) of a colorless powder of m.p.
35 289°C. UV (MeOH): 204 (4.74); 227 (4.52); [243 (4.42)]; [262 (4.21)]; 338 (3.88).

1,3-Dimethyl-6-(E)-styryllumazine-7-ylmethyl-triphenylphosphonium
bromide:

Analogous to the preceding procedure from 7-bromomethyl-
1,3-dimethyl-6-(E)-styryllumazine and triphenylphosphane in toluene by
5 heating under reflux for 1 day. Yield: 1.86 g (97%). M.p.
261°C. UV (MeOH): 204 (4.76); [221 (4.54)]; 342 (4.09);
414 (4.38).

General synthesis of 1,3-dimethyl-6-(E)-styryllumazines
10 1, 2, 3, 5:

To a solution of 1,3-dimethyl-6-(E)-styryllumazine-7-ylmethyl-
triphenylphosphonium bromide and 1,3-dimethyl-6-(E)-styryllumazine-7-ylmethyl-
triphenylphosphonium bromide (0.547 g, 1 mmole),
respectively, in MeOH (5 ml) was added sodium methoxide
15 (0.108 g, 2 mmole) and stirred at room temperature for
30 min. Then 1.5 mmole of the aromatic or heteroaromatic
aldehyde were added and stirring continued for 5 hours.
The resulting precipitate was filtered off, washed with
MeOH and purified by recrystallization from DMF/H₂O to
20 give a yellowish powder.

1,3-Dimethyl-6-(E)-styryllumazine (1):

According to the general procedure with benzaldehyde
(0.16 g). Yield: 0.124 g (42%). M.p. 238°C. UV (MeOH):
25 [220 (4.17)]; 308 (4.42); 372 (4.03).

1,3-Dimethyl-6-[(E)-2-(pyrid-3-yl)vinyl]lumazine (2):

According to the general procedure with pyridine-3-
carboxaldehyde (0.162 g). Yield: 0.195 g (66%). M.p.
30 210°C. UV (MeOH): [236 (3.92)]; 308 (4.29); 370 (3.97).

1,3-Dimethyl-6-[(E)-2-(pyrid-4-yl)vinyl]lumazine (3):

According to the general procedure with pyridine-4-
carboxaldehyde (0.162 g). Yield: 0.156 g (53%). M.p.
35 262°C. UV (MeOH): 202 (4.20); [238 (3.92)]; 307 (4.51);
370 (4.20).

6-(1,2-Dibromo-2-phenylethyl)-1,3-dimethyl-6-(E)-styryllumazine (4):

To a solution of 1,3-dimethyl-6-(E)-styryllumazine (1) (0.735 g, 2.5 mmol) in CHCl_3 (20 ml) was added bromine (0.8 g, 5 mmol) dissolved in CHCl_3 (5 ml) and then the mixture stirred at room temperature for 4 hours. It was
5 evaporated to dryness and the residue treated with MeOH to give a colorless precipitate. The solid was collected, washed with MeOH and dried in a vacuum desiccator. Yield: 1.067 g (94%). M.p. 176°C. UV (MeOH): 245 (4.18); [260 (4.10)]; 341 (3.83).

10

1,3-Dimethyl-6-[(E)-4-(phenyl)butadienyl]lumazine (5): According to the general procedure with cinnamaldehyde (0.2 g). Yield: 0.138 g (43%). M.p. 252°C (decomp.). UV (MeOH): 228 (4.02); [244 (3.97)]; 330 (4.66); 389 (4.23).

15

6-[(E)-2-methoxycarbonylethenyl]lumazine:

To a suspension of methoxycarbonylmethyl-triphenylphosphonium bromide (0.415 g, 1 mmol) in dioxane (3 ml) was added DBU (0.23 g, 1.5 mmol) and
20 stirred at room temperature for 30 min. Then 1,3-dimethyl-6-carboxaldehyde [1] (0.2 g, 0.91 mmol) was added and stirring continued for 5 hours. The precipitate was collected, washed with MeOH and dried to give a colorless crystal powder. Yield: 0.158 g (63%).
25 M.p. 211-213°C (decomp.). UV (MeOH): 202 (4.46); [256 (4.14)]; 286 (4.21); 348 (4.08).

6-(1,2-Dibromo-2-(methoxycarbonyl)ethyl)-1,3-dimethyl-6-[(E)-2-methoxycarbonylethenyl]lumazine (6):

30 To a solution of 6-[(E)-2-methoxycarbonylethenyl]lumazine (0.7 g, 2.53 mmol) in CHCl_3 (20 ml) was added bromine (0.64 g, 4 mmol) dissolved in CHCl_3 (5 ml) and then the mixture stirred at room temperature for 6 hours. It was evaporated to dryness and the residue treated with MeOH
35 to give a colorless precipitate. The solid was collected, washed with MeOH and dried in a vacuum desiccator. Yield: 0.97 g (88%). M.p. 163°C. UV (MeOH): 247 (4.16); [260 (4.08)]; 339 (3.88).

6-(2-Bromo-2-methoxycarbonyl-ethenyl)-1,3-dimethylumazine (7):

To a solution of 6 (0.1 g, 0.23 mmoles) in dioxane (20 ml) was added DBU (70 MG, 0.43 g, 0.43 mmoles) and then stirred at room temperatur for 2 hours. It was diluted with ethyl acetate (100 ml), washed with H₂O (3 x 50 ml), the organic layer separated, dried over Na₂SO₄ and then evaporated. The residue was treated with MeOH, the solid collected and purified by recrystallization from DMF to give a yellowish powder. Yield: 0.055 g (68%). M.p. 204°C. UV (MeOH): [254 (4.08)]; 285 (4.25); 360 (4.01).

6-Chlorocarbonyl-1,3-dimethylumazine:

A suspension of 1,3-dimethylumazine-6-carboxylic acid [2] (3.0 g, 12.7 mmoles) in dry toluene (80 ml) was treated with freshly distilled thionyl chloride (50 ml) under reflux for 3 hours. It was evaporated to dryness, the residue treated with dry ether, the solid collected, washed with ether and dried in a vaccuum desiccator. Yield: 3.13 g (93%). M.p. 262-264°C. UV (dioxane): 256 (4.08); [280 (4.00)]; 333 (4.03).

6-[(2-Acetyl-2-ethoxycarbonyl)acetyl]-1,3-dimethylumazine (8):

A solution of ethyl acetoacetate ethoxy-magnesium salt [3] (0.8 g, 4 mmoles) in THF (8 ml) was added dropwise to a suspension of 6-chlorocarbonyl-1,3-dimethylumazine (0.51 g, 2 mmoles) in THF (10 ml) and then the mixture stirred at room temperature for 3 days. It was evaporated and the residue treated with cold 1 N HCl (20 ml, 0-5°C). The precipitate was collected, washed with H₂O and dried in a vacuum desiccator. Purification was achieved by column chromatography (silica gel 3.5 x 12 cm) with CHCl₃/MeOH 95/5 and the first main fraction collected. After evaporation was recrystallized from toluene (12 ml) to give colorless crystals. Yield: 0.247 g (36%). M.p. 153-156°C. UV (pH 2.0): 251 (4.09); 293 (4.10); 330 (4.11).

6-[2,2-(Diethoxycarbonyl)acetyl]-1,3-dimethylumazine
(9):

To a solution of ethylmalonate ethoxy-magnesium salt [4]
(0.685 g, 3 mmoles) in THF (12 ml) was added 6-
5 chlorocarbonyl-1,3-dimethylumazine (0.51 g, 2 mmoles)
and then the mixture stirred at room temperature for 20
hours. It was evaporated, the residue treated with 1 N
HCl (20 ml) and the resulting solid collected.
Recrystallization from EtOH (40 ml) gave yellowish
10 crystals. Yield: 0.585 g (78%). M.p. 124-126°C. UV (pH
2.0): 253 (4.05); 291 (4.08); 332 (4.04).

6-(1-Methoxy-2-methoxycarbonyl)ethenyl)-1,3-
dimethylumazine (10):

15 A suspension of 6 (0.2 g, 0.46 mmoles) in dry MeOH (8 ml)
was treated with a solution of sodium (0.046 g, 2 mmoles)
in MeOH (2 ml) at room temperature with stirring for 15
min. Then NH_4Cl (0.1 g) and H_2O (10 ml) were added and the
mixture extracted with CHCl_3 (2 x 50 ml). The organic
20 layer was dried over Na_2SO_4 , evaporated and the residue
crystallized from CHCl_3 /n-hexane. Yield: 0.085 g (60%).
M.p. 160°C. UV (MeOH): 204 (4.20); 245 (4.15); 288
(4.23); 350 (3.99).

25 1,3-Dimethyl-6-[(2-nitro)ethenyl]umazine (11):

A solution of 12 (0.562 g, 2 mmoles) in pyridine (10 ml)
was cooled to 0°C and then acetic anhydride (4 ml)
dropwise added. Cooling was removed and the mixture
stirred at room temperature for 3 hours. The resulting
30 precipitate was collected, washed with H_2O and dried in a
vacuum desiccator to give a chromatographically pure
product. Yield: 0.515 g (98%). Crystallization from CHCl_3 .
M.p. 232-234°C. UV (MeOH): [239 (3.63)]; 309 (3.87); 365
(3.95).

35

6-[(1-Hydroxy-2-nitro)ethyl]-1,3-dimethylumazine (12):

To a solution of nitromethane (0.61 g, 10 mmoles) and
triethylamine (1.44 g, 10 mmoles) in MeOH (20 ml) was

added 6-formyl-1,3-dimethylalumazine [1] (2.0 g, 9 mmoles) and then the mixture stirred at room temperature for 5 hours. The precipitate was collected, washed with MeOH and ether and dried. Yield: 2.22 g (78%).

5 Recrystallization from CHCl_3 . M.p. 166-167°C. UV (MeOH): 240 (4.38); 336 (3.98); [347 (3.89)].

6-[(1-Ethylthio-2-nitro)ethyl]-1,3-dimethylalumazine (13):
To a suspension of compound 11 (0.263 g, 1 mmole) in MeOH
10 (5 ml) and H_2O (5 ml) was added ethylmercaptane (0.093 g, 1.5 mmoles) and DBU (0.2 g) and then the mixture stirred at room temperature for 1 hour. The precipitate was collected, washed and dried. Yield: 0.25 g (77%). M.p. 88°C. UV (MeOH): 203 (4.28); 240 (4.26); [262 (4.05)];
15 341 (3.89).

6-Hydroxymethyl-1,3-dimethylalumazine (14) [1]:

1,3-Dimethyl-7-[(E)-2-(pyrid-2-yl)vinyl]lumazine (15):
20 According to the general procedure with pyridine-2-carboxaldehyde (0.162 g). Yield: 0.233 g (79%). M.p. 282-283°C. UV (MeOH): 203 (4.14); 238 (4.23); 312 (3.95); 375 (4.36).

25 1,3-Dimethyl-7-[(E)-2-(pyrid-3-yl)vinyl]lumazine (16):
According to the general procedure with pyridine-3-carboxaldehyde (0.162 g). Yield: 0.195 g (66%). M.p. 264-265°C. UV (MeOH): 208 (4.45); 234 (4.43); [274 (4.09)]; 307 (4.08); 375 (4.48).

30 1,3-Dimethyl-7-[(E)-2-(pyrid-4-yl)vinyl]lumazine (17):
According to the general procedure with pyridine-4-carboxaldehyde (0.162 g). Yield: 0.215 g (73%). M.p. 307-310°C. UV (MeOH): 207 (4.12); 229 (4.01); 282 (3.79);
35 [296 (3.76)]; 372 (4.00).

1,3-Dimethyl-7-[(E)-4-(phenyl)butadienyl]lumazine (18):

According to the general procedure with cinnamaldehyde (0.2 g). Yield: 0.195 g (61%). M.p. 277-287°C (decomp.). UV (MeOH): 239 (3.79); 299 (3.66); 402 (4.15).

5 7-[1,2-Dibromo-2-(methoxycarbonyl)ethyl]-1,3-dimethylalumazine (19):

To a suspension of 7-[(E)-2-methoxycarbonylethenyl]lumazine (1.79 g, 6.5 mmol) in CHCl₃ (70 ml) was added bromine (0.7 g, 14 mmol) dissolved in CHCl₃ (10 ml) and
10 then the mixture stirred at room temperature for 3 hours. It was evaporated to dryness and the residue treated with MeOH to give a colorless precipitate. The solid was collected, washed with MeOH and dried in a vacuum desiccator. Yield: 2.34 g (77%). Crystallization from
15 EtOAc/n-hexane. M.p. 144-145°C. UV (MeOH): 240 (4.14); 343 (3.90).

7-[(E)-2-methoxycarbonylethenyl]lumazine (20):

To a suspension of methoxycarbonylmethyl-triphenylphosphonium bromide (0.415 g, 1 mmol) in
20 dioxane (5 ml) was added DBU (0.23 g, 1.5 mmol) and stirred at room temperature for 30 min. Then 1,3-dimethylalumazine-7-carboxaldehyde [1] (0.2 g, 0.91 mmol) was added and stirring continued for 20 hours.
25 The precipitate was collected, washed with MeOH and dried to give a colorless crystal powder. Recrystallization from DMF. Yield: 0.15 g (60%). M.p. 242-245°C (decomp.). UV (MeOH): 201 (4.21); 225 (4.29); 252 (4.20); 364 (4.11).

30

7-(1,2-Dibromo-2-phenylethyl)-1,3-dimethylalumazine (21):

To a solution of 1,3-dimethyl-7-(E)-styrylumazine (23) (0.735 g, 2.5 mmol) in CHCl₃ (20 ml) was added bromine (0.48 g, 3 mmol) dissolved in CHCl₃ (5 ml) and then the
35 mixture stirred at room temperature for 3 hours. It was evaporated to dryness and the residue treated with MeOH to give a colorless precipitate. The solid was collected, washed with MeOH and dried in a vacuum desiccator. Yield:

1.08 g (95%). M.p. 187-188°C. UV (MeOH): 241 (4.25); 341 (4.06).

7-(1-Bromo-2-phenyl)ethenyl-1,3-dimethylumazine (22):

5 To a suspension of 21 (0.2 g, 0.44 mmoles) in dry MeOH (4 ml) was added a solution of sodium (0.05 g, 2.2 mmoles) in MeOH (1 ml) and then the mixture stirred at room temperature for 3 hours. The precipitate was collected, washed with MeOH and dried in vacuum. Yield: 0.117 g
10 (71%). Yellowish powder from DMF. M.p. 245-246°C. UV (MeOH): 243 (4.15); 372 (4.15).

7-Benzyl-1,3-dimethylumazine:

A solution of 5,6-diamino-1,3-dimethyluracil
15 monohydrochloride [5] (2.06 g, 0.01 mole) in H₂O (50 ml) was treated with benzylglyoxal [6] (2.22 g, 0.015 moles) in EtOH (20 ml) and heated under reflux for 1 hour. It was diluted with H₂O (50 ml) and then extracted with CHCl₃ (5 x 100 ml). The organic layer was dried over Na₂SO₄,
20 evaporated and the residue purified by silica gel column chromatography with toluene/EtOAc 10/1. The main fraction was collected, evaporated and crystallized from EtOH. Yield: 1.7 g (61%). M.p. 147-148°C. UV (MeOH): 238 (4.22); 332 (4.07).

25

1,3-Dimethyl-6-(E)-styryllumazine (23):

According to the general procedure with benzaldehyde (0.16 g). Yield: 0.223 g (76%). M.p. 259-260°C. UV (MeOH): 203 (4.17); 237 (4.11); 379 (4.29).

30

7-Benzoyl-1,3-dimethylumazine (24):

A suspension of 7-benzyl-1,3-dimethylumazine (0.56 g, 2 mmoles) in H₂O (30 ml) was treated with KMnO₄ (0.6 g) and heated under reflux for 30 min. After cooling was
35 extracted with CHCl₃ (3 x 100 ml), the organic layer dried over Na₂SO₄, filtered and evaporated to dryness. Crystallization from dioxane/H₂O. Yield: 0.5 g (84%). M.p. 190-191°C. UV (MeOH): 233 (4.23); [255 (4.10)]; 347 (3.97).

7-Chloro-1,3-dimethylalumazine (25) [7].

1,3-Dimethyl-7-mercaptolumazine (26) [8].

5 1,3-Dimethyl-6,7-diphenylalumazine (27) [9].

1,3-Dimethyl-6-phenyl-7-mercaptolumazine (28):

A mixture of 7-hydroxy-1,3-dimethyl-6-phenylalumazine [5]
(2.84 g, 0.01 mole) and P_4S_{10} (3.3 g) was heated in
10 pyridine (75 ml) under reflux for 1 hour. After cooling
was diluted with H_2O (50 ml) and after standing for
several hours the yellow precipitate (28-pyridinium
salt, 3.3 g, 87%). The salt was dissolved in hot H_2O (100
ml) and acidified by HCl to pH 0. The resulting yellow
15 crystals were collected, washed and dried in the oven.
Yield: 2.22 g (74%). M.p. 145°C (decomp.). UV (MeOH): 203
(4.37); 227 (4.36); [283 (3.86)]; 370 (4.05).

7-Methoxy-1,3-dimethyl-6-phenylalumazine (29):

20 A solution 7-hydroxy-1,3-dimethyl-6-phenylalumazine [5]
(1.42 g, 0.005 moles) in 0.5 N NaOH (20 ml) and MeOH (10
ml) was treated with dimethyl sulfate (1 ml) and stirred
for 1 hour at room temperature. The resulting precipitate
was collected, washed and dried in the oven. Yield: 1.26
25 g (81%). M.p. 194°C. UV (MeOH): 205 (4.53); [240 (4.08)];
281 (4.22); 343 (4.23).

7-Chloro-1,3-dimethyl-6-phenylalumazine (30):

A mixture of 7-hydroxy-1,3-dimethyl-6-phenylalumazine [5]
30 (2.84 g, 0.01 mole) and NH_4Cl (1 g) was heated in $POCl_3$
under reflux for 36 hours. It was evaporated to a syrup,
ice was added and stirred with a glass rod till a
precipitate was formed. The solid was collected, washed
with H_2O , dried and then recrystallized from MeOH. Yield:
35 2.36 g (78%). M.p. 180°C. UV (MeOH): 204 (4.47); 249
(4.23); 273 (4.24); 350 (4.05).

6-Benzoyl-7,8-dihydro-1,3-dimethyl-7-(4-methoxyphenyl)lumazine (31a):

A solution of 6-benzoyl-1,3-dimethyl-7-(4-methoxyphenyl)lumazine (0.2 g, 0.68 mmol) in dry 1,2-dichloroethane (20 ml) was treated with AlCl_3 (0.4 g, 3 mmol) and freshly distilled anisole (10 ml, 92 mmol) at room temperature and stirred for 24 hours. Then ice (50 g) was added, the aqueous phase extracted with CHCl_3 (3 x 50 ml), the organic phase washed with 2% NaHCO_3 solution (50 ml) and H_2O (50 ml), dried over Na_2SO_4 and evaporated in high vacuum to remove excess of anisole. The residue was treated with toluene (50 ml) to obtain a yellow precipitate. Recrystallization from $\text{EtOH}/\text{H}_2\text{O}$ 1/1 gave yellow crystals. Yield: 0.176 g (65%). M.p. 240-244°C (decomp.). UV (MeOH): 254 (4.25); [270 (4.21)]; 406 (4.08).

6-Benzoyl-1,3-dimethyl-7-(4-methoxyphenyl)lumazine (31):

A suspension of 6-benzoyl-7,8-dihydro-1,3-dimethyl-7-(4-methoxyphenyl)lumazine (31a) (0.3 g, 0.74 mmol) in dioxane (40 ml) was treated at room temperature with 1% KMnO_4 solution (10 ml) by dropwise addition with stirring. After 30 min the excess of KMnO_4 was reduced by NaHSO_3 , the MnO_2 filtered off, washed with warm EtOH (3 x 20 ml) and then the united organic phases evaporated to dryness. The residue was purified by silica gel chromatography with $\text{CHCl}_3/\text{MeOH}$ (25/1). The main fraction was collected, evaporated and the solid recrystallized from EtOAc with charcoal. Yield: 0.175 g (59%). M.p. 255-257°C. UV (MeOH): 253 (4.24); 367 (4.10).

6-Benzoyl-7,8-dihydro-1,3-dimethyl-7-phenyllumazine (32a):

Analogous to procedure 31a from 6-benzoyl-1,3-dimethyl-7-phenyllumazine (0.2 g, 0.68 mmol) and benzene (15 ml). Yield: 0.21 g (83%). UV (MeOH): 254 (4.26); 407 (4.12).

6-Benzoyl-1,3-dimethyl-7-phenyllumazine (32):

Analogous to procedure 31 from 6-benzoyl-7,8-dihydro-1,3-dimethyl-7-phenyllumazine (32a) (0.3 g, 0.78 mmoles).
Yield: 0.18 g (62%). M.p. 185-187°C. UV (MeOH): 252 (4.39); [290 (4.08)]; 349 (4.16).

5

7-Methoxy-1,3-dimethyl-6-styryllumazine (33):

To a suspension of compound 4 (0.2 g, 0.44 mmoles) in dry MeOH (6 ml) was added DBU (0.2 ml, 1.34 mmoles) and then stirred at room temperature for 2 hours. The precipitate
10 was filtered off, washed with MeOH and dried in a vacuum desiccator. Yield: 0.134 (94%). Crystallization from DMF. M.p. 271-272°C. UV (MeOH): [232 (4.11)]; 306 (4.36); 375 (4.38).

15 1-Methyl-6,7-diphenyllumazine (34) [10].

7-Hydroxy-3-methyl-6-phenyllumazine (35) [5].

7-Hydroxy-1,6-diphenyllumazine (36).

20 To a suspension of 6-diamino-5-nitroso-1-phenyluracil [11] (2.32 g, 0.01 moles) in H₂O (50 ml) and EtOH (20 ml) was reduced catalytically with PtO₂/H₂ in a shaking apparatus till about 450 ml of hydrogen was consumed. The mixture was heated, the catalyst filtered off and the
25 filtrate treated with ethyl phenylglyoxylate (2.5 g, 0.014 mmoles) by heating under reflux for 30 min. The warm solution was acidified by HCl to pH 0 and the resulting precipitate collected after cooling. Recrystallization from DMF. Yield: 2.59 (78%). M.p.
30 330°C. UV (MeOH): 204 (4.54); [222 (4.37)]; 284 (4.17); 346 (4.25).

7-Hydroxy-3,6-dimethyl-1-phenyllumazine (37) [12]:

35 7-Hydroxy-6-phenyl-1,3-di-n-propyllumazine (38):

A suspension of 5,6-diamino-1,3-di-n-propyluracil (1.13 g, 0.005 moles) in H₂O (30 ml), EtOH (5 ml) and AcOH (2 ml) was treated with ethyl phenylglyoxylate (1.25 g,

0.007 mmoles) and heated under reflux for 30 min forming a brownish oil. After cooling was acidified by HCl to pH 0 whereby the oil solidified. Filtration and recrystallization from EtOH/H₂O gave yellowish needles.
5 Yield: 1.28 g (75%). M.p. 245°C. UV (MeOH): 212 (4.30); [243 (4.01)]; 284 (4.02); 349 (4.19).

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Materials and methods

Various models may be used for testing an immunosuppressive effect. In vivo, for example, different transplantation models are available. They are strongly influenced by different immunogenicities, depending on the donor and recipient species used and depending on the nature of the transplanted organ. The survival time of transplanted organs can thus be used to measure the suppression of the immune response. In vitro, there exist also various models. The most used are lymphocyte activation tests. Usually activation is measured via lymphocyte proliferation. Inhibition of proliferation thus always means immunosuppression under the experimental conditions applied. There exist different stimuli for lymphocyte activation:

- coculture of lymphocytes of different species (MLR = mixed lymphocyte reaction): lymphocytes expressing different minor and major antigens of the HLA-DR type (= alloantigens) activate each other non-specifically.
- CD3 assay: here there is an activation of the T-lymphocytes via an exogenously added antibody (OKT3). This antibody reacts against the CD3 molecule located on the lymphocyte membrane. This molecule has a costimulatory function. The interaction anti-CD3 (= OKT3)-CD3 results in T-cell activation which proceeds via the Ca^{2+} /calmodulin/calcineurin system and can be inhibited by CyA.
- CD28 assay: here specific activation of the T-lymphocyte goes also via an exogenously added antibody against the CD28 molecule. This molecule is also located on the lymphocyte membrane, and delivers strong costimulatory signals. This activation is Ca^{2+} -independent and thus cannot be inhibited by CyA.

35 Reagents

All derivatives were dissolved in 0.5 ml DMSO and further diluted in culture medium before use in in vitro

experiments. The culture medium consisted of RPMI-1640 + 10% FCS.

Mixed Lymphocyte Reaction

5 Peripheral blood mononuclear cells (PBMC) were isolated from heparinized peripheral blood by density gradient centrifugation over Lymphoprep (Nycomed, Maorstua, Norway). Allogeneic PBMC or EBV-transformed human B cells [RPMI1788 (ATCC name CCL156)] which
10 strongly express B7-1 and B7-2 were used as stimulator cells after irradiation with 30 Gy. MLR was performed in triplicate wells. After 5 days incubation at 37°C, 1 μ Ci [³H]-thymidine was added to each cup. After a further 16 hours incubation, cells were harvested and counted in a
15 β -counter.

The percent suppression of proliferation by drugs was counted using the formula:

$$\begin{aligned} \text{Percent inhibition} &= (\text{cpm+drugs}) - \text{cpm Cult. Med} \\ &\frac{\text{-----}}{(\text{cpm-drugs}) - \text{cpm Cult. Med}} \times 100 \end{aligned}$$

T cell purification

T cells were purified by removing non-T cells. Briefly, monocytes were removed by cold agglutination. The resulting lymphoid cells were further purified by a cell enrichment immunocolumn [Celect Human T (Biotex, Edmonton, Alberta, Canada)] by a process of negative selection. More than 95% of the B cells were removed with
30 this procedure. After depletion, the resulting T cell preparation was highly purified explaining these cells could not be activated by PHA or rIL-2 alone at concentrations capable of stimulating RBMC prior to deletion.

35

Measurements of T cell proliferations induced by anti-CD3 mAb + PMA or anti-CD28 mAb + PMA

Highly purified T cells ($10^6/\text{ml}$) were stimulated by immobilized anti-CD3 or anti-CD28 mAb in the presence of PMA. Anti-CD3 mAb (CLB-CD3; CLB, Amsterdam, The Netherlands) were fixed on the 96-microwell plates by incubating the wells with 50 μl of mAb solution (1/800 dilution in culture medium). Anti-CD28 mAb (CLB-CD28; CLB, Amsterdam, The Netherlands) 50 μl (1/650 dilution in culture medium) was added directly to the wells. Further, 20 μl PMA (Sigma, St. Louis, MO, USA) solution (final concentration: 0.5 ng/ml) was added. Subsequently, 20 μl of immunosuppressants were added by serial dilution in triplicate wells. Finally 100 μl of the T cell suspension ($10^6/\text{ml}$) was added. After 48-hour incubation at 37°C in 5% CO_2 20 μl BrdU (100 μM solution) (Cell Proliferation Elisa, Boehringer-Mannheim Belgium) was added to each well. After a further overnight incubation the T cell proliferation was measured using a colorimetric immunoassay for qualification of cell proliferation based on measurements of the incorporation of BrdU during DNA synthesis. The optical density (OD) was measured by a Behring EL311 plate reader at 450 nm (reference wavelength: 690 nm). The percent suppression of proliferation by drugs was counted using the formula:

$$\text{Percent inhibition} = \frac{(\text{OD+drugs}) - (\text{OD Cult. Med.})}{(\text{OD-drugs}) - (\text{OD Cult. Med.})} \times 100$$

In vitro immunosuppressive effect of Pteridine Derivatives as measured with the MLR and with tests involving polyclonal T cell proliferation induced by anti-CD3 mAb + PMA or anti-CD28 mAb + PMA (table II)

- In the table II column II shows the IC_{50} values of the various substances in the MLR. The IC_{50} value represents the lowest concentration of the substances that resulted in a 50% suppression of the MLR.

- Column III shows the IC_{50} value of the various substances for the anti-CD3 mAb + PMA pathway and row IV the IC_{50} values of the various substances for the

anti-CD28 mAb + PMA pathway.

- As a comparison the values of other immunosuppressants: CsA, FK506, Rapamycin, Leflunomide and Mycophenolic acid methatroxate (MTX) and 5-Fluoro-uracil (5-FU) in table III are given as well.

Whole Blood Assay (WBA)

WBA is a lymphoproliferation assay performed in vitro but using lymphocytes present in whole blood, taken from animals that were previously given test substances in vivo. Hence it reflects the in vivo effect of substances as assessed with an in vitro read-out assay.

Rats: inbred, male 6- to 8-weeks old R/A rats weighing \pm 200 g were used as recipients.

Drug administration: Pteridine derivatives were dissolved in DMSO and further diluted with PBS. Products were given orally in different concentrations 2 times a day for 2 days. To perform the experiments, 6-8 hours after the last administration 1 ml of blood is taken by heart puncture after ether anesthesia and anticoagulated with 100 U/ml of preservative free heparine.

Whole Blood Assay: This assay was performed as we described previously [Use of the Methylxanthine Derivatives A802715 in Transplantation Immunology. II In vitro Experiments. (Yuan Lin, et al., Transplantation 1997, 63, No. 12, 1734-1738)].

Heparinized whole blood was diluted (1:25) with complete RPMI medium and stimulated with 15 μ g/ml of concanavalin A (Con A) in triplicate wells in 96-well microtiter plates at 37 °C and 5% CO₂. After 96-h culture, proliferation was determined by measuring the incorporation (cpm) of [³H]-thymidine.

The Con A induced proliferation of lymphocytes taken from rats receiving the test substances (exp) was compared with that from rats receiving only the solvent (con). The percent suppression was calculated as follows:

5 Results

No.	%suppression	Administration of drugs	Blood taken after:
28	57%	80 mg/kg/d	8 h
34	63%	2x/d 2d 40 mg/kg/d 2x/d 2d	8 h

10 First, most of the pteridine classes (I) according to the invention contain compounds with a clear suppressive effect in the MLR (mixed lymphocyte reaction). The MLR is considered as an in vitro analogue of the transplant rejection as it is based on the recognition of allogeneic MHC (major histocompatibility antigens) on the stimulator leucocytes, by responding lymphocytes. Various established immunosuppressive drugs are known to suppress the MLR, and were also shown in this description.

20 From these data it can be deduced that the pteridine derivatives are effective in clinical situations where other immunosuppressants are active as well.

25 These include the prevention and/or treatment of organ transplant rejection, the prevention and/or treatment of both rejection and the occurrence of graft-versus-host-disease after BM transplantation; the prevention and/or treatment of autoimmune diseases including diabetes mellitus, multiple sclerosis, glomerulonephritis, rheumatoid arthritis, psoriasis, systemic diseases such as vasculitis; scleroderma, 30 polymyositis, autoimmune endocrine disorders (thyroiditis), ocular diseases (uveitis), inflammatory bowel diseases (Crohn's disease, colitis ulcerosa), autoimmune liver diseases (autoimmune hepatitis, primary

biliary cirrhosis) autoimmune pneumonitis and auto-immune carditis.

Whereas cyclosporine A and FK506 are only active in the anti-CD3 + PMA test, the pteridine derivatives according to the invention were active, not only in the anti-CD3 + PMA but also in the anti-CD28 + PMA test. It has been shown that the latter is Ca-calmodulin resistant, and resistant to CsA and FK506. The anti-CD28 + PMA pathway has also been called the cosignal pathway and is important to induce energy and even tolerance in T cells. Moreover, representative compounds have been found to be active in an whole blood assay.

Under the term "organ" in the description is understood all organs or parts of organs (even several) in mammals, in particular humans, for example kidney, heart, skin, liver, muscle, cornea, bone, bone marrow, lung, pancreas, intestine or stomach.

After organ transplantation, rejection of the transplanted organ by the recipient occurs (host-versus-graft reaction). After bone marrow transplantation, also rejection of the host by the grafted cell may occur (graft-versus-host reaction). Rejection reactions mean all reactions of the recipient body or of the transplanted organ which in the end lead to cell or tissue death in the transplanted organ or adversely affect the functional ability and viability of the transplanted organ or adversely affect the functional ability and viability of the transplanted organ or the recipient. In particular, this means acute and chronic rejection reactions.

Auto-immune disorders include, inter alia, systemic lupus erythematosus, rheumatoid arthritis, psoriasis, pemphigus, atopic dermatitis, myositis, multiple sclerosis, nephrotic syndrome (in particular glomerulonephritis), ulcerative colitis or juvenile diabetes.

An additive or synergetic effect of pteridine derivatives and other immunosuppressants may be

anticipated. This may be especially, although not exclusively the case for combinations with CyA or FK 506 as the latter are not suppressive in the aCD28 pathway of T cell activation (table III) whereas most pteridine derivatives are.

The invention further relates to the use of cyclosporin A or FK506 or Rapamycine and at least one pteridine derivative according to the invention for the production of a pharmaceutical composition for inhibiting the replication of viruses such as picorna-, toga-, bunya-, orthomyxo-, paramyxo-, rhabdo-, retro-, arena-, hepatitis B-, hepatitis C-, hepatitis D-, adeno-, vaccinia-, papilloma-, herpes-, varicella-zoster-virus or human immunodeficiency virus (HIV); or for treating of cancer such as lung cancers, leukaemia, ovarian cancers, sarcoma, Kaposi's sarcoma, meningioma, colon cancers, lymph node tumors, glioblastoma multiforme, prostate cancers or skin carcinoses.

The invention further relates to the use of cyclosporin A or FK506 or rapamycin and at least one pteridine derivative of the general formula (I) for the production of a pharmaceutical composition for the treatment of human after organ transplantation or of (auto)immune disorders.

Hence, the advantage to associate pteridine with other immunosuppressants may be that, first, the therapeutic spectrum of action of the individual components is quantitatively and qualitatively broadened. Secondly that it allows, by means of a dose reduction without reduced efficacy but with increased safety, that the treatment of immune disorders which were hitherto no indication for immunosuppressive therapy as a result of side effects may be considered. At the same time, the therapy costs can be decreased to an appreciable extent.

As a comparison, known pteridine derivatives are submitted to the same test conditions as the pteridine derivatives of the invention. These compounds and the results thereof are given in table IV and show no

particular immunosuppressive activity.

In all tables

0: concentration > 151 μM

+: concentration range 16-150 μM

5 ++: concentration range 1-15 μM

+++: concentration range < 1 μM

The skilled person will appreciate that the preparation according to the invention may contain the pteridine compounds over a broad content range depending on the contemplated use of the preparation. Generally, the content of the preparation is within the range of 0.01-50 wt.%, preferably within the range of 0.01-10 wt.%, more preferably within the range of 0.1-10 wt.%, and most preferably within the range of 0.1-5 wt.%. Accordingly, the preparation may be used in a dosing regime which is suitable for most contemplated pharmaceutical utilities.

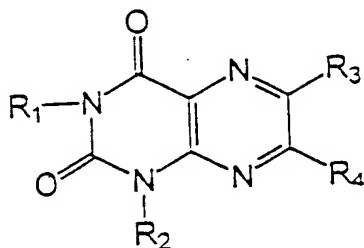
The preparation according to the invention may be used as such or in combination with any acceptable carrier material, excipient or diluent.

The preparation according to the invention may be administered orally or in any other suitable fashion. Oral administration is preferred and the preparation may have the form of a tablet, aqueous dispersion, dispersable powder or granule, emulsion, hard or soft capsule, syrup, elixir or gel. The dosing forms may be prepared using any method known in the art for manufacturing these pharmaceutical compositions and may comprise as additives sweeteners, flavoring agents, coloring agents, preservatives and the like. Carrier materials and excipients may include calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, binding agents and the like. The preparation may be included in a gelatin capsule mixed with any inert solid diluent or carrier material, or has the form of a soft gelatin capsule, in which the ingredient is mixed with a water or

oil medium. Aqueous dispersions may comprise the preparation in combination with a suspending agent, dispersing agent or wetting agent. Oil dispersions may comprise suspending agents such as a vegetable oil.

CLAIMS

1. Pharmaceutical composition for the treatment of autoimmune disorders and/or for the treatment or prevention of transplant-rejections comprising a pteridine derivative of general formula:



wherein:

R_1 and R_2 are independently hydrogen; aliphatic saturated or unsaturated; straight or branched carbon chain with 1 to 7 carbon atoms; substituted or unsubstituted aryl or alkylaryl substituents, whereby the carbon atoms may be oxidized represented by alcohol or carbonyl function or carboxylic acids and their esters;

R_3 and R_4 are independently hydrogen, hydroxyl, halogen, alkyl, haloalkyl, alkoxy, wherein alkyl and the alkyl group may be branched or straight and contains one or four carbon atoms, formyl and derivatives such as hydroxylamino conjugates and acetals, cyano, carboxylic acids and carboxyl acid derivatives such as esters and amides, sulfhydryl, amino, alkylamino, cycloalkylamino, alkenylamino, alkynylamino, benzylamino, hydroxylalkylamino, morfolinoalkylamino, fenylhydrazino, morfoline, piperidine, mercaptobenzyl, mercaptoalkyl, cysteinyl ester, styryl, substituted or unsubstituted aromatic ring; aromatic or heterocyclic substituent substituted with an aliphatic spacer between the

pteridine ring and the aromatic substituent of 1 to 4 carbon atoms, whereby said spacer may contain an alcohol function, carbonyl function, halogen, ether, and may be saturated or unsaturated; branched or straight, saturated or unsaturated aliphatic chain of 1 to 7 carbon atoms which may contain one or more functions chosen from the group comprising carbonyl, alcohol, ether, carboxyester, nitro, thioalkyl, halogen or a pharmaceutically acceptable salt thereof; and

10

X and Y are independently oxygen or sulfur or a pharmaceutical salt thereof, and a pharmaceutically acceptable carrier.

15

2. Pharmaceutical composition according to claim 1, wherein:

20

R_1 and R_2 are independently hydrogen, methyl, ethyl, propyl, isopropyl, cyclopropyl allyl and other alifatic and alicyclic saturated and unsaturated substituents with 1 to 5 carbon atoms;

25

3. Pharmaceutical composition according to claim 1-2, wherein:

30

R_4 and R_5 are independently hydrogen; hydroxyl; halogen; alkoxy whereby the alkyl group being one to four carbon atoms; cyano; carboxyl acids and carboxyl acid derivatives such as esters and amides; sulfhydryl; substituted or unsubstituted aromatic or heterocyclic ring having as substituents one or more of following functions chosen from the group comprising: halogen, alkoxy, hydroxy, amino, alcohol, ether, nitro, alkyl, alkenyl, haloalkyl and haloalkenyl, cyano, carboxyl acids and derivatives; aromatic or heterocyclic substituent with an aliphatic spacer between the pteridine ring and the aromatic substituent of 1 to 4 carbon atoms, this

35

spacer may contain an alcohol function, carbonyl function, carboxyl, nitro, halogen, ether and may be saturated or unsaturated, branched or unbranched; branched or straight, saturated or unsaturated aliphatic chain of 1 to 7 carbon atoms which may contain one or more of following functions chosen from the group comprising: carbonyl, alcohol, ether, acetal, amino, imino, amino acid, carboxylester, cyano, nitro, thioalkyl, halogen; or a pharmaceutically acceptable salt thereof.

4. Pharmaceutical composition according to claim 1-3, wherein:

15 R_1 and R_2 are methyl.

5. Pharmaceutical composition according to claim 4, wherein R_4 is hydrogen.

6. Pharmaceutical composition according to claim 5, wherein R_3 is haloethyl or haloethylene substituted by phenyl or carboxylic acid ester; or is ethyl or ethylene substituted by nitro.

7. Pharmaceutical composition according to claim 4, wherein R_3 is hydrogen.

8. Pharmaceutical composition according to claim 7, wherein R_4 is haloethyl or haloethylene substituted by phenyl or carboxylic acid ester; or is chloro.

9. Pharmaceutical composition according to claim 4, wherein R_3 is selected from cyano, carboxylic acid ester or dihalo methyl; and R_4 is selected from chloro, amino C_4 -alkyl, aminophenyl, thiomethylphenyl, thiomethyl-carboxylic acid ester.

10. Pharmaceutical composition according to any of the previous claims 1-9, wherein the pteridine derivative is a compound selected from the group comprising:

- 35 - 1,3-Dimethyl-6-(E)-styryllumazine (1)
- 1,3-Dimethyl-6-[(E)-2-(pyrid-3-yl)vinyl]lumazine
(2)

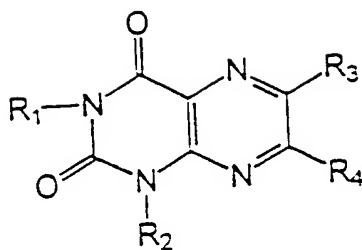
- 1,3-Dimethyl-6-[(E)-2-(pyrid-4-yl)vinyl]lumazine
(3)
- 6-(1,2-Dibromo-2-phenylethyl)-1,3-dimethylumazine
(4)
- 5 - 1,3-Dimethyl-6-[(E)-4-(phenyl)butadienyl]lumazine
(5)
- 6-(1,2-Dibromo-2-(methoxycarbonyl)ethyl)-1,3-
dimethylumazine (6)
- 6-(2-Bromo-2-methoxycarbonyl-ethenyl)-1,3-
10 dimethylumazine (7)
- 6-[(2-Acetyl-2-ethoxycarbonyl)acetyl]-1,3-
dimethylumazine (8)
- 6-[2,2-(Diethoxycarbonyl)acetyl]-1,3-
dimethylumazine (9)
- 15 - 6-(1-Methoxy-2-methoxycarbonyl)ethenyl)-1,3-
dimethylumazine (10)
- 1,3-Dimethyl-6-[(2-nitro)ethenyl]lumazine (11)
- 6-[(1-Hydroxy-2-nitro)ethyl]-1,3-dimethylumazine
(12)
- 20 - 6-[(1-Ethylthio-2-nitro)ethyl]-1,3-
dimethylumazine (13)
- 6-Hydroxymethyl-1,3-dimethylumazine (14)
- 1,3-Dimethyl-7-[(E)-2-(pyrid-2-yl)vinyl]lumazine
(15)
- 25 - 1,3-Dimethyl-7-[(E)-2-(pyrid-3-yl)vinyl]lumazine
(16)
- 1,3-Dimethyl-7-[(E)-2-(pyrid-4-yl)vinyl]lumazine
(17)
- 1,3-Dimethyl-7-[(E)-4-(phenyl)butadienyl]lumazine
(18)
- 30 - 7-[1,2-Dibromo-2-(methoxycarbonyl)ethyl)-1,3-
dimethylumazine (19)
- 7-[(E)-2-methoxycarbonylethenyl]lumazine (20)
- 7-(1,2-Dibromo-2-phenylethyl)-1,3-dimethylumazine
(21)
- 35 - 7-(1-Bromo-2-phenyl)ethenyl-1,3-dimethylumazine
(22)
- 1,3-Dimethyl-6-(E)-styryllumazine (23)

- 7-Benzoyl-1,3-dimethylalumazine (24)
- 7-Chloro-1,3-dimethylalumazine (25)
- 1,3-Dimethyl-7-mercaptopalumazine (26)
- 1,3-Dimethyl-6,7-diphenylalumazine (27)
- 5 - 1,3-Dimethyl-6-phenyl-7-mercaptopalumazine (28)
- 7-Methoxy-1,3-dimethyl-6-phenylalumazine (29)
- 7-Chloro-1,3-dimethyl-6-phenylalumazine (30)
- 6-Benzoyl-7,8-dihydro-1,3-dimethyl-7-(4-methoxyphenyl)alumazine (31a)
- 10 - 6-Benzoyl-1,3-dimethyl-7-(4-methoxyphenyl)alumazine (31)
- 6-Benzoyl-7,8-dihydro-1,3-dimethyl-7-phenylalumazine (32a)
- 6-Benzoyl-1,3-dimethyl-7-phenylalumazine (32)
- 15 - 7-Methoxy-1,3-dimethyl-6-styrylumazine (33)
- 1-Methyl-6,7-diphenylalumazine (34)
- 7-Hydroxy-3-methyl-6-phenylalumazine (35)
- 7-Hydroxy-1,6-diphenylalumazine (36)
- 7-Hydroxy-3,6-dimethyl-1-phenylalumazine (37)
- 20 - 7-Hydroxy-6-phenyl-1,3-di-n-propylalumazine (38).

11. Pharmaceutical composition containing a pteridine derivative according to claim 1-10 and a compound selected from the group comprising cyclosporine A, substituted xanthines, tacrolimus (FK506), Rapamycin (RPM), Leflunomide, Mofetil, adrenocortical steroids, cytotoxic drugs and antibody compositions as a combined preparation for simultaneous, separate or sequential use in the treatment of auto-immune disorders or of the prevention and/or treatment of transplant-rejection.

30 12. Method for treating auto-immune disorders or transplant-rejections in a subject by administering an effective amount of a pharmaceutical composition of claims 1-11.

13. Compound having the formula:



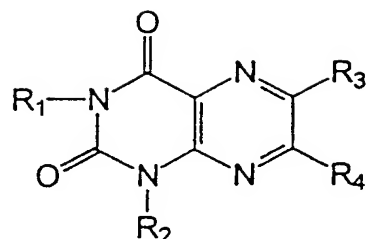
wherein:

R₁, R₂, R₃ and R₄ are defined in claims 1-10.

10 14. Use of a compound as defined in claim 10 for the treatment of autoimmune disorders and/or the treatment and/or prevention of transplant rejections.

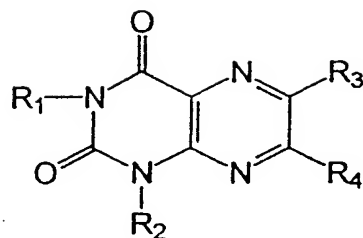
15 15. Method for selecting potent immunosuppressive agents based on the determination of the three parameters MLC, ACD₃ and ACD₂₈.

Table I



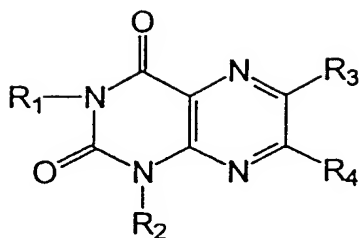
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1	CH ₃	CH ₃	—CH=CH—	H
2	CH ₃	CH ₃	—CH=CH—	H
3	CH ₃	CH ₃	—CH=CH—	H
4	CH ₃	CH ₃	—CHBr—CHBr—	H
5	CH ₃	CH ₃	—(CH=CH) ₂ —	H
6	CH ₃	CH ₃	—CHBr—CHBr—COOCH ₃	H
7	CH ₃	CH ₃	—CH=CH—COOCH ₃	H
8	CH ₃	CH ₃	—CO—CH—COOEt COCH ₃	H
9	CH ₃	CH ₃	—CO—CH—COOEt COOEt	H
10	CH ₃	CH ₃	—C=CH—COOCH ₃ OCH ₃	H
11	CH ₃	CH ₃	—CH=CH—NO ₂	H
12	CH ₃	CH ₃	—CHOH—CHNO ₂	H
13	CH ₃	CH ₃	—CH—CH ₂ NO ₂ SC ₂ H ₅	H
14	CH ₃	CH ₃	CH ₂ OH	H

Table I

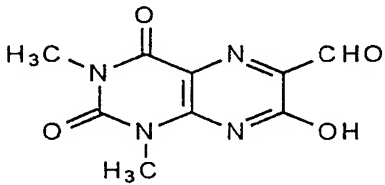
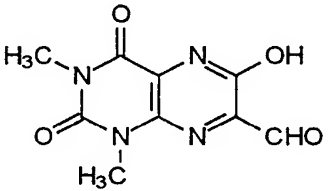
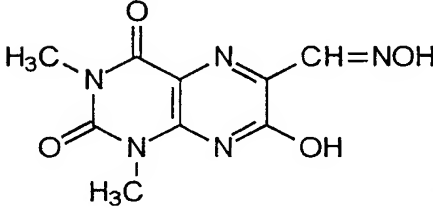
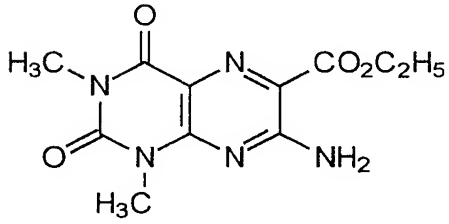
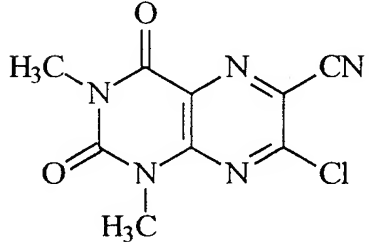


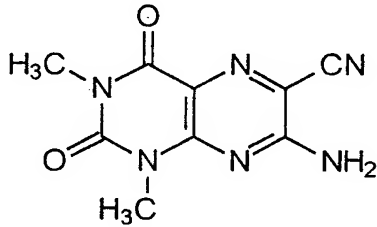
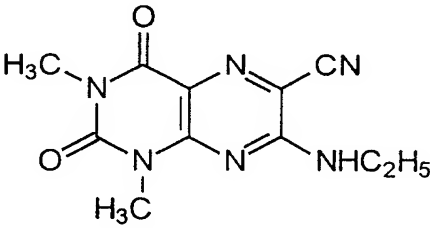
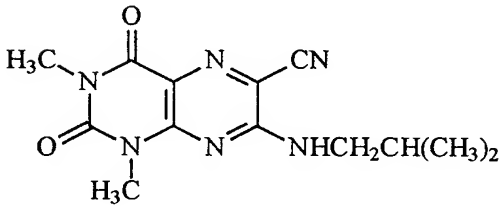
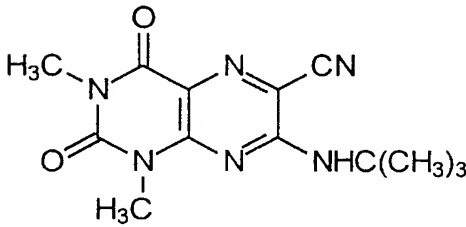
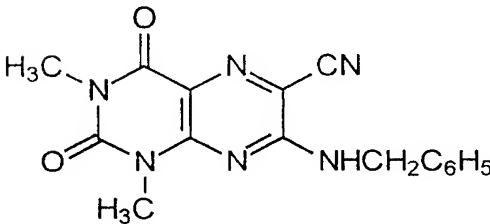
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16	CH ₃	CH ₃	H	
17	CH ₃	CH ₃	H	
18	CH ₃	CH ₃	H	
19	CH ₃	CH ₃	H	
20	CH ₃	CH ₃	H	
21	CH ₃	CH ₃	H	
22	CH ₃	CH ₃	H	
23	CH ₃	CH ₃	H	
24	CH ₃	CH ₃	H	
25	CH ₃	CH ₃	H	Cl
26	CH ₃	CH ₃	H	SH

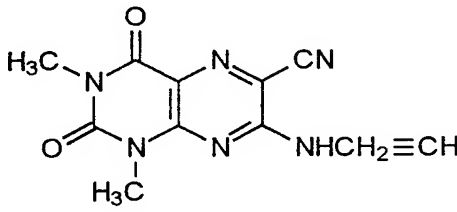
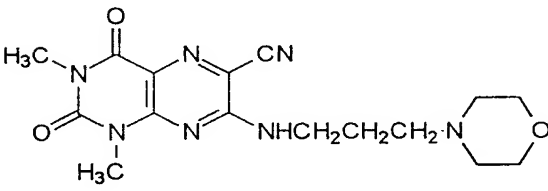
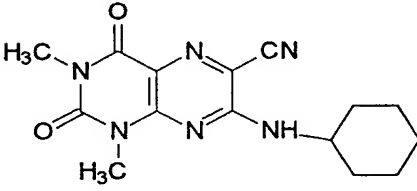
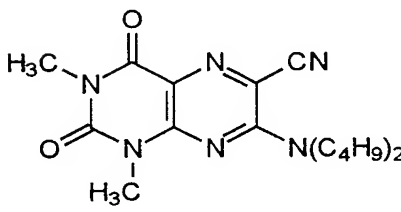
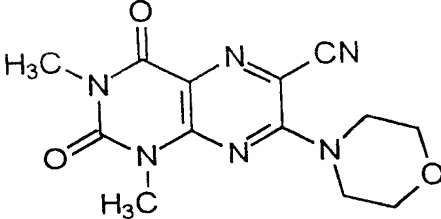
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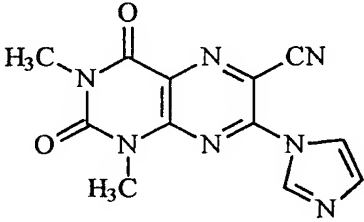
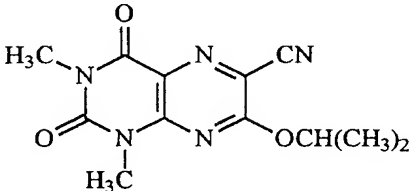
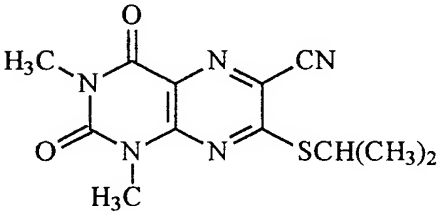
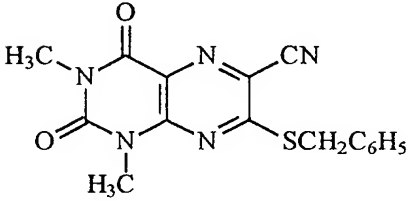
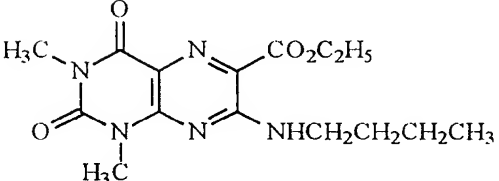


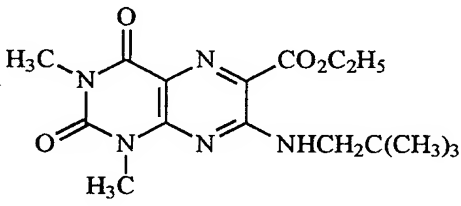
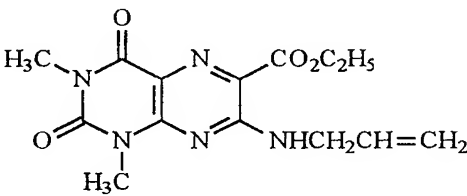
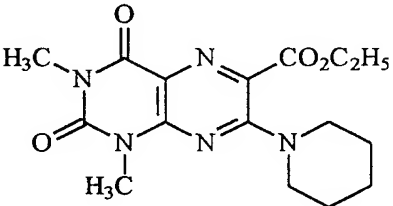
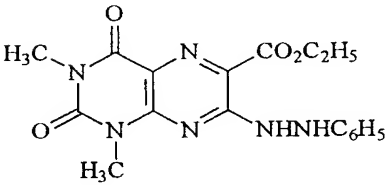
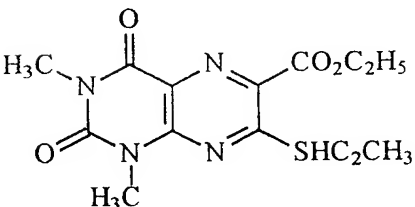
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28	CH ₃	CH ₃		SH
29	CH ₃	CH ₃		OCH ₃
30	CH ₃	CH ₃		Cl
31	CH ₃	CH ₃		
32	CH ₃	CH ₃		
33	CH ₃	CH ₃		OCH ₃
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35	CH ₃	H		OH
36	H			OH
37	CH ₃		CH ₃	OH
38	Pr	Pr		OH

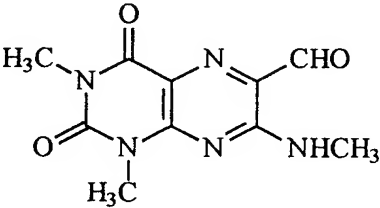
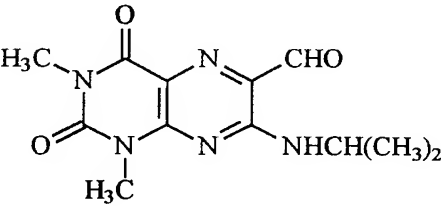
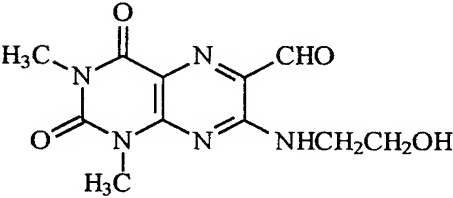
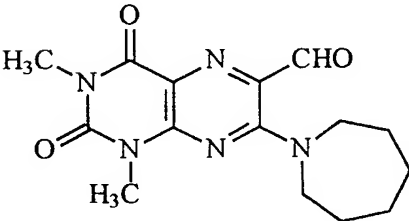
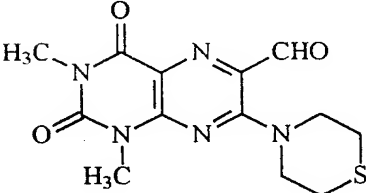
Compound n°	MLR	aCD3	aCD2 8
39			
	0	++	++
40			
	+	+	+
41			
	0	0	0
42			
	0	0	0
43			
	+	++	++

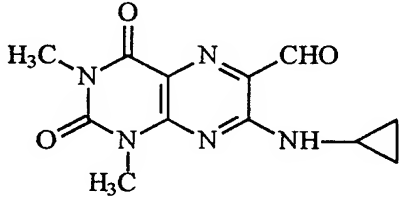
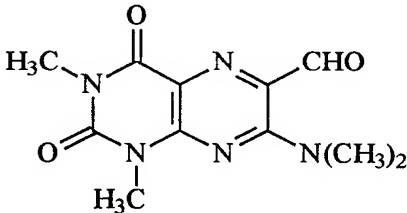
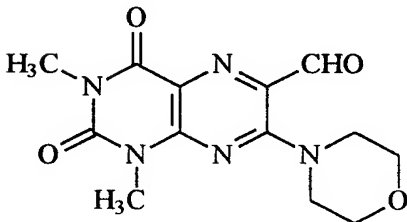
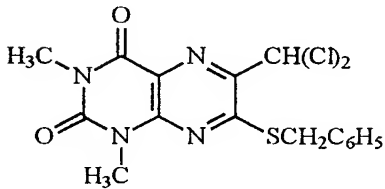
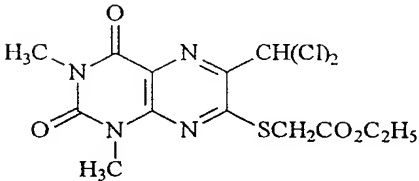
Compound n°	MLR	aCD3	aCD2 8
49			
	0	0	0
50			
	0	0	0
51			
	+	+	+
52			
	+	+	0
53			
	+	+	+

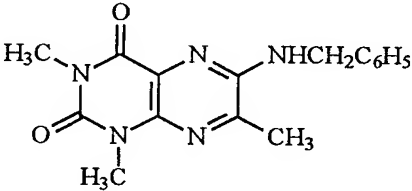
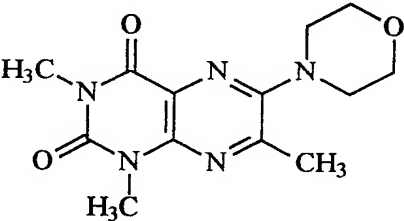
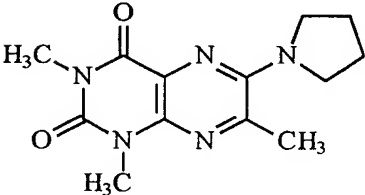
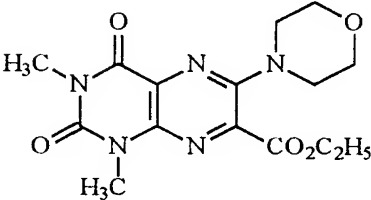
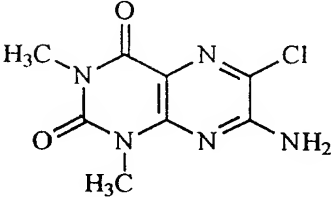
Compound n°	MLR	aCD3	aCD2 8
54			
	+	+	+
55			
	0	0	+
56			
	++	+	+
57			
	++	0	+
58			
	0	0	0

Compound n°	MLR	aCD3	aCD2 8
59			
	+	++	++
60			
	0	0	0
61			
	+	+	+
62			
	0	++	++
63			
	++	+	+

Compound n°	MLR	aCD3	aCD2 8
64			
	+	+	+
65			
	+	+	+
66			
	+	+	+
67			
	+	+	++
68			
	+	+	+

Compound n°	MLR	aCD3	aCD2 8
69			
	+	0	+
70			
	+	+	+
71			
	+	+	+
72			
	+	+	+
73			
	+	+	+

Compound n°	MLR	aCD3	aCD2 8
74			
	+	+	+
75			
	0	0	+
76			
	0	+	+
77			
	+	++	++
78			
	+	++	++

Compound n°	MLR	aCD3	aCD2 8
79			
	+	+	+
80			
	0	+	+
81			
	+	+	+
82			
	+	+	+
83			
	0	0	0

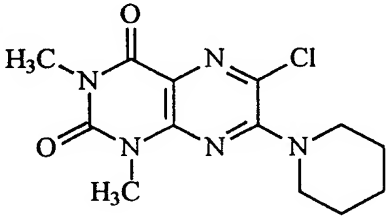
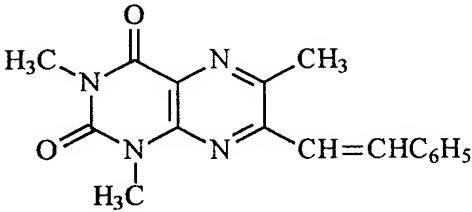
Compound n°	MLR	aCD3	aCD2 8
84			
	0	+	+
85			
	+	+	+

Table II

Compound n°	MLR	aCD3	aCD28
1	75	> 200	> 200
2	30	110	110
3	20	150	150
4	15	3.4	1.55
5	75	140	140
6	12	0.55	0.08
7	4.2	0.58	0.08
8	> 200	80	20
9	> 200	125	90
10	70	80	70
11	12	0.8	0.4
12	90	25	15
13	4.5	0.35	0.09
14	> 200	> 200	25

Table II

IC₅₀ in μ M of pteridine derivative

Compound n°	MLR	ACD3	aCD28
15	125	125	80
16	20	100	60
17	75	125	100
18	30	110	80
19	1.9	0.5	0.5
20	15	12	12
21	12	0.6	0.1
22	2.7	2.4	2.7
23	50	115	115
24	120	120	80
25	12	0.5	0.1
26	60	35	25

Table II

IC₅₀ in μ M of pteridine derivative

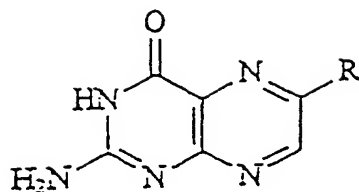
Compound n°	MLR	aCD3	aCD28
27	> 200	85	100
28	25	25	25
29	40	100	70
30	3.75	0.08	0.07
31	150	90	90
32	> 200	75	75
33	20	> 200	> 200
34	35	25	25
35	55	20	25
36	> 200	75	110
37	65	180	> 200
38	120	80	70

Table III

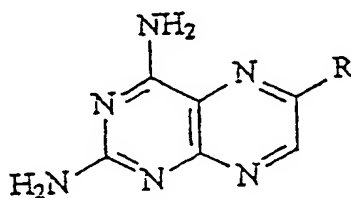
I.S.	IC50		
	Immunosuppressant		
	MLR	aCD3	aCD28
CyA	20 nM	50 nM	N.S.
FK506	1 nM	1 nM	N.S.
Rapamycin	1 nM	1 nM	1 nM
Leflunomide	25 μ M	15 μ M	20 μ M
Mofetil	<0.5 μ M	50 nM	50 nM
MTX	10 μ M	>200 μ M	> 200 μ M
5-FU		50 μ M	17 μ M

N.S. = not suppressive even not in the highest
Concentration

Table IV



R	MLR	aCD3	aCD28
CH ₂ OOCOCH ₃	>200	>200	>200
CH ₂ OC ₆ H ₅	>200	>200	>200
CH ₂ NHCH ₃	>200	>200	>200
CH ₂ N(CH ₃) ₂	>200	>200	>200
CH ₂ S CH ₃	>200	>200	>200



R	MLR	aCD3	aCD28
CH ₂ S CH ₃	>200	>200	>200
CH ₂ O CH ₂ (CH ₃) ₂	>200	>200	>200
CH ₂ O CH ₃	>200	>200	>200
CH ₂ O CH ₂ CH ₂ CH ₃	>200	>200	>200
CH ₂ NH CO CH (CH ₃) ₂	>200	>200	>200

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
10 August 2000 (10.08.2000)

PCT

(10) International Publication Number
WO 00/45800 A3

(51) International Patent Classification⁷: **A61K 31/505**,
38/13, 31/52, 31/535, 31/57, C07D 475/02, A61P 37/06,
37/00

(21) International Application Number: PCT/EP00/00938

(22) International Filing Date: 2 February 2000 (02.02.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/118,295 2 February 1999 (02.02.1999) US
60/118,235 2 February 1999 (02.02.1999) US
60/118,282 2 February 1999 (02.02.1999) US

(71) Applicant (*for all designated States except US*): **K.U. LEUVEN RESEARCH & DEVELOPMENT** [BE/BE];
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(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **WAER, Mark, Jozef, Albert** [BE/BE]; Waversebaan 263, B-3001 Heverlee (BE). **HERDEWIJN, Piet, André, Maurits, Maria** [BE/BE]; Olivierstraat 21, B-3111 Rotselaar/Wezemaal (BE). **PFLEIDERER, Wolfgang, Eugen** [DE/DE]; Lindauerstrasse 47, D-78464 Konstanz (DE).

(74) Agent: **PRINS, Hendrik, Willem**; Arnold & Siedsma, Sweelinckplein 1, NL-2517 GK The Hague (NL).

(81) Designated States (*national*): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

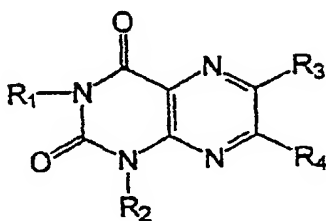
Published:

— with international search report

(88) Date of publication of the international search report:
10 January 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: IMMUNOSUPPRESSIVE EFFECTS OF PTERIDINE DERIVATIVES



(I)

(57) Abstract: The invention relates to a pharmaceutical composition for the treatment of autoimmune disorders and/or for the treatment or prevention of transplant-rejections comprising a pteridine derivative of general formula (I) especially a lumazine optionally combined with a second active agent.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/00938

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/505 A61K38/13 A61K31/52 A61K31/535 A61K31/57
C07D475/02 A61P37/06 A61P37/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 52948 A (UNIV CALIFORNIA) 26 November 1998 (1998-11-26)	1-10, 13
A	page 3, line 1-13; figure 10 page 5, line 11-19 page 4, line 12-20 page 7, line 15-22 page 31-34; claims 1-4, 8, 20-23 ---	11, 12, 14
X	WO 96 20710 A (UNIV CALIFORNIA) 11 July 1996 (1996-07-11)	1-10, 13
A	page 16-19; claims 6-9, 18 page 23, line 4-26; claims 22, 25, 26, 30 --- -/-	12, 14



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search

29 May 2000

Date of mailing of the international search report

22.02.01

Name and mailing address of the ISA

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KANBIER D.T.

INTERNATIONAL SEARCH REPORT

Inter. Application No

PCT/EP 00/00938

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 // (A61K31/505, 31:435), (A61K31/505, 31:42), (A61K38/13, 31:505),
(A61K31/52, 31:505), (A61K31/535, 31:505), (A61K31/57, 31:505)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	COTTAM ET AL: "Substituted Xanthines, Pteridinediones, and Related Compounds as Potential Antiinflammatory Agents. Synthesis and Biological Evaluation of Inhibitors of Tumor Necrosis Factor-alpha" J. MED. CHEM., vol. 39, no. 1, 1996, pages 2-9, XP000906858	1-3, 12, 13
A	page 2, left-hand column; table 1 page 5, right-hand column, paragraph 2 --- -/--	4, 5, 10, 14

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

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KANBIER D.T.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/00938

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 94 11001 A (CELL THERAPEUTICS INC) 26 May 1994 (1994-05-26) page 3, line 9-12; claims 1,6,12 page 5, line 19 -page 6, line 27 page 9, line 7 -page 14, line 32 page 17, line 10-16 page 21 page 27, line 12-26 ----	1,3, 11-14 2,4,5,7
X A	US 5 641 783 A (KLEIN J PETER ET AL) 24 June 1997 (1997-06-24) column 25 column 4, line 13-21 column 9-14; claims 1-3,11,18 ----	1,3, 12-14 2,4,5,7
X	WO 94 22449 A (CELL THERAPEUTICS INC) 13 October 1994 (1994-10-13) page 1, line 7-9; claims 11,19 page 4, line 9-11 page 8, line 10-27 page 11, line 11-24 ----	1,12-14
X	WO 95 31469 A (US HEALTH) 23 November 1995 (1995-11-23) page 3, line 8-16 page 3, line 8-16; claim 1 ----	13
X	WO 95 13075 A (CELL THERAPEUTICS INC) 18 May 1995 (1995-05-18) claims 1,6 ----	13
A	BUU-HOI, N. P. ET AL: "Phthalonimides (1,3,4-trioxo-1,2,3,4-tetrahydroisoquinoli nes) of potential biological interest" J. HETEROCYCL. CHEM., vol. 5, no. 4, 1968, pages 545-547, XP000906859 the whole document ----	1,12-14
A	WO 96 10568 A (MERCK & CO INC) 11 April 1996 (1996-04-11) page 30-31 page 117, line 18-25 -----	1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/00938

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 12-15 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-14

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-14

First and further medical claims for pteridinediones and their application in treating autoimmune disorders and/or treating/preventing transplant rejections

2. Claim : 15

Method for the selection of immunosuppressive agents by means of determination based on the parameters MLC, ACD3 and ACD28.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-5, 7 and 1-13 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the entire claimed scope is impossible.

Furthermore, the terminology of Claims 1, 2 and 3 (e.g. "substituted", "heterocyclic substituent") renders the scope of these claims unclear in the sense of Article 6 PCT.

Finally, present claim 1 contains a passage which is obscure in the sense that it is contradictory, even in view of the appropriate passages in the description (page 4, lines 6-7):

"X and Y are independently salt thereof", page 27, lines 10-11.

There is no reference anywhere in the present application to X and Y.

Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds claimed in Claims 6 and 8-10, with due regard to the idea underlying the application.

Present claim 11 relates to an extremely large number of possible compounds to be combined with the pteridine derivatives of the application. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the entire claimed scope is impossible.

Furthermore, the terms "substituted xanthines", "adrenocortical steroids", "antibody compositions" and "cytotoxic drugs" render the scope of claim 11 unclear in the sense of Article 6 PCT, since it is not immediately and exhaustively apparent to a skilled person which compounds/compositions are comprised therein.

Consequently, the search has been carried out for those parts of the claim which appear to be supported and disclosed, namely those parts relating to combinations of pteridine derivatives with compounds specifically disclosed in claim 11 itself, on page 23, lines 6-8; page 2, lines 8-10, lines 18-19 and lines 25-26 of the description (cyclosporin A, Rapamycin, FK506; leflunomide, mofetil; pentoxifylline, A802715), with due regard to the idea underlying the application.

Claims searched completely: 6, 8-10, 14.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 00/00938

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 00/00938

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		EP 0783498 A	16-07-1997
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